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Long-chain *n*-3 fatty acids as an essential link between musculoskeletal and cardio-metabolic health in older adults

Oliver C. Witard[1]*, Emilie Combet[2] and Stuart R. Gray[2]
[1]*Centre for Human and Applied Physiological Sciences, School of Basic and Medical Biosciences, Faculty of Life Sciences and Medicine, King's College London, London, UK*
[2]*College of Medical, Veterinary and Life Sciences, University of Glasgow, Scotland, UK*

This narrative review aims to critically evaluate scientific evidence exploring the therapeutic role(s) of long-chain *n*-3 PUFA in the context of ageing, and specifically, sarcopenia. We highlight that beyond impairments in physical function and a lack of independence, the age-related decline in muscle mass has ramifications for cardio-metabolic health. Specifically, skeletal muscle is crucial in regulating blood glucose homeostasis (and by extension reducing type 2 diabetes mellitus risk) and providing gluconeogenic precursors that are critical for survival during muscle wasting conditions (i.e. AIDS). Recent interest in the potential anabolic action of *n*-3 PUFA is based on findings from experimental studies that measured acute changes in the stimulation of muscle protein synthesis (MPS) and/or chronic changes in muscle mass and strength in response to fish oil-derived *n*-3 PUFA supplementation. Key findings include a potentiated response of MPS to amino acid provision or resistance-based exercise with *n*-3 PUFA in healthy older adults that extrapolated to longer-term changes in muscle mass and strength. The key mechanism(s) underpinning this enhanced response of MPS remains to be fully elucidated, but is likely driven by the incorporation of exogenous *n*-3 PUFA into the muscle phospholipid membrane and subsequent up-regulation of cell signalling proteins known to control MPS. In conclusion, multiple lines of evidence suggest that dietary *n*-3 PUFA provide an essential link between musculoskeletal and cardio-metabolic health in older adults. Given that western diets are typically meagre in *n*-3 PUFA content, nutritional recommendations for maintaining muscle health with advancing age should place greater emphasis on dietary *n*-3 PUFA intake.

***n*-3: Cardio-metabolic disease risk: Healthy ageing: Anabolic resistance**

The amount and type of dietary fat consumed is widely recognised to play an important role in determining metabolic health in human subjects[1]. Fatty acids are hydrocarbon chains of varying lengths with a carboxyl group and methyl group at opposing ends. The presence of one or several double bonds in (unsaturated) fatty acids impacts on their conformation, as well as their function. Very long-chain or long-chain *n*-3 PUFA are a class of fatty acids distinguished by two or more double bonds at the methyl end of the carbon chain. The most abundant species of *n*-3 PUFA are EPA, DHA and α-linolenic acid. EPA consists of a C_{20} chain with five double bonds, DHA a C_{22} chain with six double bonds, and α-linolenic acid a C_{18} chain with three double bonds. As human subjects are unable to endogenously synthesise α-linolenic acid, it is defined as an essential

Abbreviation: MPS, muscle protein synthesis.
***Corresponding author:** Oliver C. Witard, email oliver.witard@kcl.ac.uk

fatty acid that must be acquired from the diet. The most commonly cited health benefit associated with increasing dietary *n*-3 PUFA intake relates to a reduction in CVD risk[2], as mediated by improvements in the regulation of blood pressure, vascular function and cardiac rhythm, although recent evidence has cast doubt on some of these claims. Recent evidence also proposes a physiological role for *n*-3 PUFA in regulating skeletal muscle protein metabolism[3] and, by extension, muscle mass[4], muscle strength[5] and muscle function. Other papers in this volume focus on the impact of dietary fatty acids on liver fat content and metabolism[6] and regional/ectopic fat depots in human adipose tissue. This brief review focuses on human skeletal muscle tissue and, specifically, the role of *n*-3 PUFA in the context of sarcopenia and sarcopenic obesity. Our narrative is divided into three distinct themes. First, we identify food sources of *n*-3 PUFA and their consumption at the population level. Next, we provide a holistic overview of the importance of skeletal muscle tissue for cardio-metabolic health, physical function and disease prevention in human subjects or man. Finally, we critique available evidence that evaluates the role of *n*-3 PUFA as a component of non-pharmacological strategies designed to tackle sarcopenia and sarcopenic obesity.

Dietary sources of long-chain *n*-3 PUFA

Commonly consumed food sources rich in *n*-3 PUFA include oily fish such as mackerel, sardines, trout and salmon (Fig. 1). In comparison, canned tuna contains a lower *n*-3 PUFA content and is no longer categorised as an oil-rich fish source. While other non-fish food sources such as walnuts also contain *n*-3 PUFA, the *n*-3 PUFA are shorter chain (often α-linolenic acid) which, in human subjects, are poorly converted to EPA and then DHA through processes of elongation and desaturation. Interestingly, this conversion is poorer in men than in women[7].

Dietary guidelines in the UK recommend two, 140 g, portions of fish per week, one of which should be of oily source[8]. However, the latest National Diet and Nutrition Survey[9] indicates that, on average, adults aged 19–64 years consume only 56 g oily fish on a weekly basis (excluding canned tuna), while older adults aged 65+ consume 84 g oily fish per week. While the average oily fish intake falls alarmingly short of this 140 g recommendation, also noteworthy is the median intake for both age groups is 0 g per week, with the majority of UK adults avoiding dietary intake of oily fish altogether. Evidence from the EPIC-Norfolk study highlights that cod liver oil (a source of *n*-3 PUFA) was the most popular supplement consumed by 32 % of men and 45 % of women[10]. However, it is worth noting that over-the-counter fish oil preparations do not always contain the dose advertised on the label, and that the fatty acids can often be extensively oxidised, compromising their proposed biological function[11,12].

The Scientific Advisory Committee on Nutrition recommends a long-chain *n*-3 PUFA intake of 450 mg/d. In comparison, UK intakes of EPA and DHA are estimated at 244 mg/d (131 mg/d from oil-rich fish)[13], with potentially lower intakes in ethnic minority groups. Hence, there is ample scope to explore strategies to increase *n*-3 PUFA intakes in the UK diet, potentially through enrichment strategies targeting foods such as dairy and meat (especially poultry)[13], with a view to improving cardio-metabolic health. While *n*-3 PUFA intake is low in the Western population, *n*-6 PUFA consumption remains comparatively high, through regular intake of seed oils and food products. It is understood that the ratio of *n*-6 : *n*-3 PUFA has recently shifted from a balanced 1:1 to about 20:1, with implications for metabolism, specifically the production of pro-inflammatory molecules, such as prostaglandins and leukotrienes[14].

Importance of skeletal muscle tissue for cardio-metabolic health and physical function

The term cardio-metabolic risk describes a family of risk factors of metabolic origin that increase the risk of developing CVD such as CHD, stroke, type 2 diabetes mellitus and chronic kidney disease. Skeletal muscle tissue plays a crucial, albeit often underappreciated, role in maintaining cardio-metabolic health and offsetting morbidities commonly associated with advancing age[15]. Accounting for about 40 % of total body mass[16], skeletal muscle is described as a plastic tissue that is capable of (mal)adaptation to physical (in)activity and diet. As the primary site of blood glucose disposal, skeletal muscle accounts for about 80 % of postprandial glucose uptake[17]. Low muscle mass is associated with a reduced RMR that can lead to the accumulation of fat mass[15]. Therefore, the maintenance of skeletal muscle mass over the lifecourse is critical in regulating blood glucose homeostasis and reducing the risk of type 2 diabetes mellitus, as well as other associated cardio-metabolic diseases. In addition, skeletal muscle serves as the body's primary storage site for amino acids and, during starvation or in the context of conditions such as AIDS by providing gluconeogenic precursors that are crucial for survival[18]. Beyond metabolic health, it is widely recognised that skeletal muscle is crucial in preserving physical function, mobility and ultimately independence during older age.

An inevitable, albeit partially modifiable, feature of the ageing process concerns the progressive decline in skeletal muscle mass, strength and function. Muscle atrophy begins as early as the fourth decade of life[19], continues at a rate of about 1 % of total muscle mass per year until age 70 years[20], and increases to about 1·5 % of total muscle mass per year above age 80 years[21]. Alarmingly, the decline in muscle strength with advancing age typically exceeds the decline in muscle mass, with annual declines of 3–4 % in strength commonly reported[22]. Once the decline in muscle strength and muscle mass falls below critical thresholds, older adults are classified as sarcopenic[23]. This condition is associated with a 2–3 fold increase in the risk of falling,

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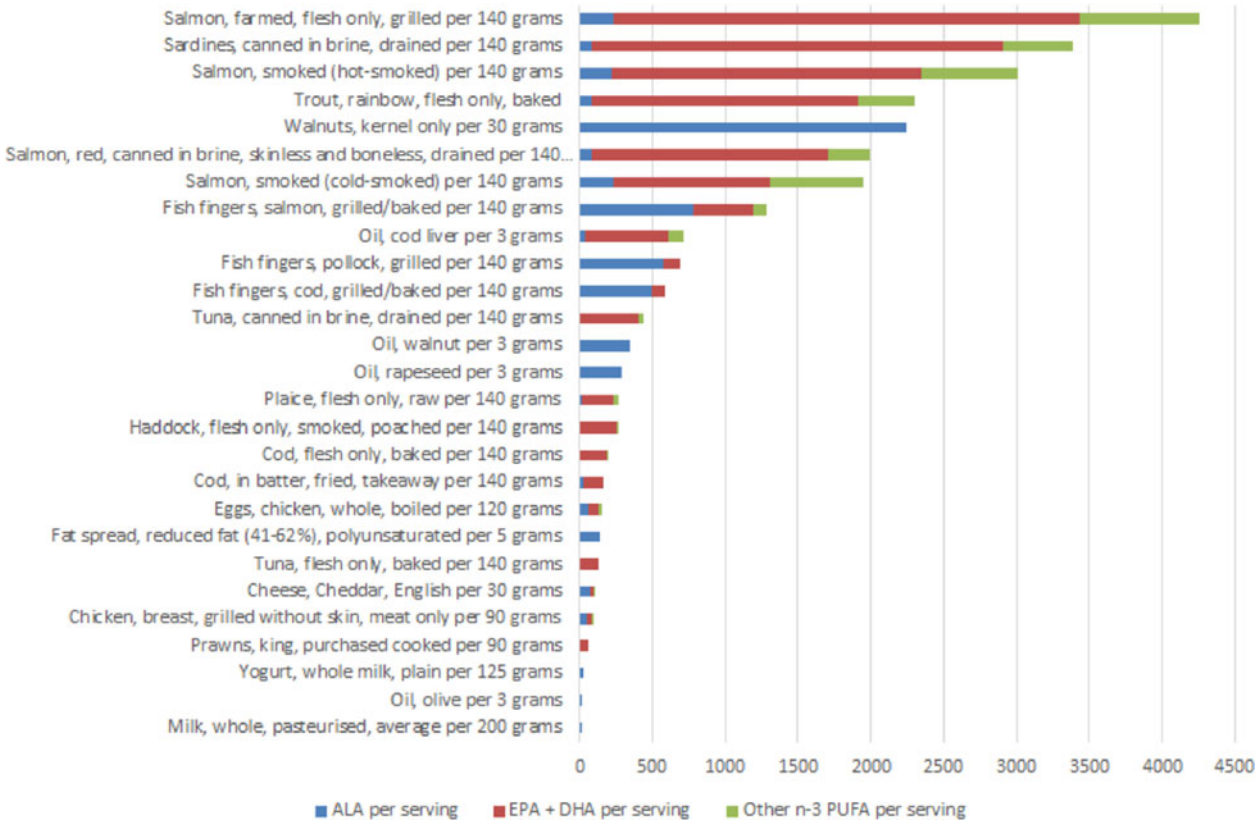

Fig. 1. Commonly consumed *n*-3 PUFA-rich food sources in the UK diet. Data extracted from the composition of foods integrated dataset (CoFID)[70]. *n*-3 PUFA, very long-chain *n*-3 PUFA; ALA, α-linoleic acid.

bone fractures, loss of independence and increased mortality[24,25]. According to a recent report, additional health and social care costs associated with sarcopenia in the UK are currently estimated to be £2·5 billion annually[26].

In 2016, sarcopenia was recognised as an independent geriatric condition, with its own International Classification of Disease code. Compounding this progressive loss of functional ability, the age-related decline in muscle mass and strength is associated with an increased cardio-metabolic health risk. In this regard, a recent study demonstrated that low muscle strength was associated with an increased risk of all-cause mortality and mortality from CVD, cancer and respiratory disease[27]. Similarly, low muscle strength has been associated with a higher incidence of type 2 diabetes mellitus[27], with findings more equivocal for low muscle mass[28,29]. Furthermore, the increased risk of CVD mortality observed in patients with type 2 diabetes mellitus is attenuated in those individuals with greater grip strength[30]. Taken together, these observational data provide compelling evidence that the maintenance of muscle mass and strength with advancing age is critical for the management of cardio-metabolic health risk.

The decline in muscle mass with advancing age often occurs in concert with an increase in fat mass. This age-related phenomenon is referred to as sarcopenic obesity. It is well established that obesity independently increases the risk of many cardio-metabolic health outcomes such as myocardial infarction, stroke, some cancers and all-cause mortality[31–33]. Evidence also suggests that when sarcopenia and obesity are combined, the debilitating effects are additive. For example, whilst sarcopenia and obesity are independently associated with an increased risk of all-cause mortality (sarcopenia hazard ratio 1·41 (95 % CI 1·22, 1·63)) and obesity hazard ratio 1·21 (95 % CI 1·03, 1·42) compared to lean non-sarcopenic individuals, all-cause mortality risk is even greater (hazard ratio 1·72 (95 % CI 1·35–2·18)) in sarcopenic obese men[34]. Therefore, it seems prudent to target the maintenance/increase of muscle mass, strength and function alongside the loss of fat mass to optimal levels in older adult populations. Before establishing targeted interventions to offset the age-related decline in muscle mass and increase in fat mass, it is important to understand the causal mechanism(s) that underpin the decline in muscle mass with advanced age.

Causal mechanisms that underpin the decline in muscle mass, strength and function with age

Although sarcopenia affects about 10–30 % of community-dwelling men and women aged 60+ worldwide, the underlying pathology of this clinical condition is not fully understood. Clearly, the underlying cause of

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sarcopenia is multifactorial, with interconnected and complex contributing factors. In terms of muscle atrophy, contributing factors include, but are not limited to, chronic low-grade inflammation, elevated levels of oxidative stress, DNA damage, mitochondrial dysfunction and hormonal changes[35]. Ultimately however, from a metabolic standpoint, the decline in muscle mass with advanced age is underpinned by a state of negative muscle protein balance.

Two possible metabolic drivers of negative muscle protein balance exist. First, an impaired stimulation of muscle protein synthesis (MPS), defined as the rate by which freely available amino acids in the blood or muscle amino acid pools are incorporated into functional muscle protein. Secondly, an up-regulation of muscle protein breakdown, defined as the rate by which muscle protein is degraded into amino acid precursors. There is a general consensus that basal, post-absorptive rates of MPS are comparable between young and older adults[36–38]. In contrast, several studies have reported suppressed postprandial rates of MPS in response to amino acid feeding in older adults compared with their younger counterparts[39]. The concept of this so-called anabolic resistance has been conceived from this observation and describes the age-related impairment in response of MPS to ingesting a meal-like (about 20 g) quantity of protein and/or other typically robust anabolic stimuli such as mechanical loading, i.e. structured exercise training. At the molecular level, this age-related impairment in MPS appears to be mediated by a dysregulation in the Akt-mTOR (mechanistic target of rapamycin) cell signalling cascade that controls the rate-limiting translation initiation step of MPS[40]. As such, anabolic resistance is widely regarded as one of the key drivers of sarcopenia. Moreover, as further evidence of the interplay between mechanisms underlying sarcopenia, animal studies have demonstrated that low-grade inflammation, which is particularly prevalent in sarcopenic obese individuals, impairs the stimulation of MPS in response to food intake[41]. Hence, there is a clear biological rationale to establish non-pharmacological lifestyle-friendly interventions that target overcoming both anabolic resistance and low-grade inflammation in older adults.

In practical terms, the progressive decline in muscle mass and strength is exacerbated by periods of muscle disuse[42,43]. Examples of skeletal muscle disuse range in duration and severity from short-term periods of limb immobilisation caused by injury (i.e. accidental falls) to longer-term periods of bedrest inflicted by illness and/or cardio-metabolic disease. A reduction in physical activity, as typically quantified by step count, provides another important, albeit less extreme, example of muscle disuse. Accordingly, age-related anabolic resistance is exacerbated by reducing physical activity levels[44], limb immobilisation[42,45] and bedrest[46]. Moreover, recent evidence suggests that age-related anabolic resistance is further exacerbated in overweight and/or obese older adults[47] (Fig. 2) and in response to a period of high-fat feeding[48]. Thus, it follows that optimising diet and lifestyle strategies for maintaining muscle health is of critical importance in sarcopenic older adults. In this

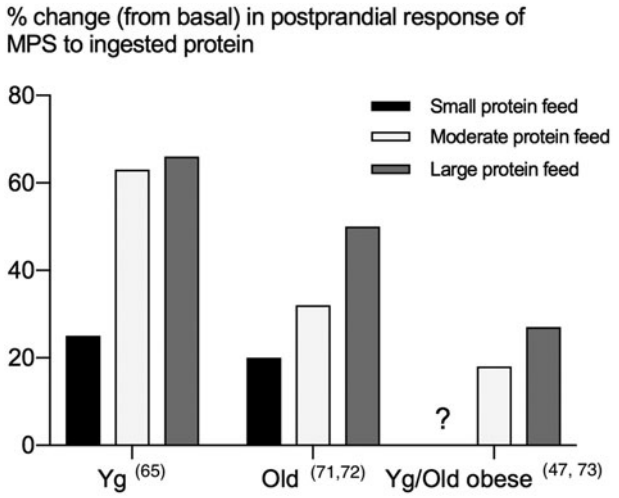

Fig. 2. Theoretical model of muscle 'anabolic resistance' associated with ageing and obesity. Data are generated from citations denoted by number in parentheses:[65] young (18–35 years) adults ingested 10, 20 or 40 g whey protein;[71] older (65–75 years) ingested 10 g whey protein;[72] older (65–75 years) ingested 20 or 40 g soya protein;[47] older (66–73 years) obese (BMI > 30) adults ingested 15 g milk protein isolate;[73] young (23–30 years) obese (BMI > 33) adults ingested 170 g pork containing 36 g protein. Small protein feed, 10 g protein; moderate protein feed, 20 g protein, large protein feed, 36–40 g protein. MPS, muscle protein synthesis; Yg, young adults; Old, older adults.

regard, given the potent anti-inflammatory properties of *n*-3 PUFA[49] and recent evidence that *n*-3 PUFA exhibit anabolic properties[50,51], the role of dietary *n*-3 PUFA intake in combating sarcopenia has received considerable recent attention.

Diverse biological roles of long-chain *n*-3 fatty acids

A key determinant of physiological function at the cellular level includes the fatty acid composition of the phospholipid cell membrane. Membrane fatty acid composition is modulated by metabolic, genetic and hormonal factors, and of particular relevance to this review, dietary intake of fatty acids. As detailed earlier, the western diet is generally rich in *n*-6 PUFA (e.g. linoleic acid) relative to *n*-3 PUFA. This pattern is reflected in the constituent fatty acid composition of cell membranes which typically range from 10 to 20 % for *n*-6 PUFA and 2 to 5 % for *n*-3 PUFA[52]. The membrane composition of *n*-3 PUFA can be elevated in a dose-dependent manner by dietary intake of *n*-3 PUFA[53]. Functionally, the most important *n*-3 PUFA are EPA and DHA and many research studies have investigated the physiological properties of EPA/DHA, primarily due to their potential to reduce inflammation[52].

Whilst inflammation is an important defence mechanism of the immune system to protect human subjects from infection, unresolved pathological inflammation can result in damage and disease. For example, and as detailed previously, low-grade chronic inflammation

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has been implicated in the aetiology of sarcopenia but also many cardio-metabolic conditions. There is a host of research demonstrating that increasing *n*-3 PUFA intake serves to reduce inflammation, as reviewed previously[52]. As inflammation has been associated with many cardio-metabolic conditions, it has been suggested that *n*-3 PUFA supplementation may be of therapeutic use. For example, early observational studies in Inuits demonstrated that even though this population consumed very high-fat diets, the prevalence of heart disease was low, with this inverse relationship attributed to the high dietary *n*-3 PUFA intake[54,55]. In contrast, a recent meta-analysis demonstrated that increasing EPA and DHA consumption has minimal, or no effect, on mortality or cardiovascular health[56], with the authors calling for a halt in further studies until ongoing large trials are fully reported.

In addition to their anti-inflammatory properties and role in regulating immune function, *n*-3 PUFA exhibit other physiological roles due to their incorporation into all cell types. Therefore, it is not surprising that the physiological roles of EPA and DHA are not limited to the immune system. For example, DHA is vital for fetal brain and retinal development given the high propensity for DHA incorporation in the brain and retinal membrane phospholipids that are crucial for the functional development of these tissues[57]. Since the recent observation that EPA and DHA supplementation results in an increased incorporation of EPA and DHA in muscle cells[51], there has been a growing interest in the physiological effects of such a change for muscle health with advancing age.

Role of long-chain *n*-3 fatty acids in prevention and treatment of sarcopenia

Dietary *n*-3 PUFA have received considerable recent attention in the context of optimising diet for the management of sarcopenia. Extending early epidemiological data which found that fatty fish consumption was positively associated, in a dose–response manner, with grip strength[58], two seminal experimental studies in healthy young, middle-aged and older adults sparked interest in the potential muscle anabolic action of *n*-3 PUFA[59,60]. These proof-of-principle, acute metabolic, studies were conducted under controlled laboratory conditions and measured rates of MPS under basal (fasted and rested) and simulated fed conditions before and after 8 weeks of fish oil (4 g/d) derived *n*-3 PUFA supplementation (1·86 g EPA, 1·50 g DHA daily). Amino acids and insulin were infused intravenously to partially mimic the ingestion of a protein-rich mixed macronutrient meal. Whereas the basal response of MPS was not modulated by *n*-3 PUFA, the feeding-induced increase in MPS was potentiated by 30–60 % after 8 weeks of fish oil supplementation compared with before supplementation[59,60].

Perhaps surprisingly, at least from a mechanistic standpoint, in these studies[59,60] no changes in TNFα or C-reactive protein concentrations, as systemic markers of inflammation, were observed over the 8-week period of fish oil supplementation. However, the phosphorylation status of intramuscular cell signalling proteins known to up-regulate MPS (e.g. mTORC1-p70S6k1) was potentiated in response to simulated feeding following dietary fish oil supplementation. Consistent with this observation, our laboratory reported an increase in the proportion of *n*-3 PUFA, specifically EPA, in the muscle cell following 4 weeks of fish oil (5 g/d) derived *n*-3 PUFA supplementation in healthy young men[51]. Such structural modifications to the muscle cell membrane were also associated with an increased phosphorylation of mTORC1 (a nutrient-sensitive intramuscular cell signalling protein and focal adhesion kinase) a mechanically sensitive kinase known to regulate MPS. Therefore, the primary causal mechanism that appears to underpin the anabolic action of *n*-3 PUFA relates to modifying the lipid profile of the muscle phospholipid membrane and subsequently up-regulating the activity of intracellular signalling proteins, rather than an anti-inflammatory response.

In recent years, we[61,62] and others[63] have extended these acute metabolic studies to investigate the anabolic and/or anti-catabolic potential of *n*-3 PUFA in young and older adults using more physiologically relevant experimental study designs (Fig. 3). Rather than the intravenous infusion of amino acids and insulin to simulate feeding, anabolic stimuli included either an orally ingested dose of intact protein, a standardised mixed macronutrient meal and/or a resistance exercise session(s) administered over a period of 1–4 d. Informed by our *in vitro* experiment with fully differentiated C2C12 cells whereby EPA, rather than DHA, was shown to both up-regulate the MPS response to a leucine stimulus and down-regulate muscle protein breakdown[64], these studies have primarily administered high-dose (3–5 g/d) fish oil supplements that are rich in EPA content. Accordingly, Lalia *et al.*[63] reported that fish oil supplementation (3·9 g/d) augmented the acute response of MPS to conducting a single bout of resistance exercise alongside feeding a protein-containing meal by about 30 % in older adults. As a note of caution, data values for MPS (expressed as fractional synthesis rate) were remarkably high in this study, calling into question the validity of these findings.

However, study findings regarding the influence of *n*-3 PUFA supplementation on postprandial rates of MPS have been equivocal, which may be attributed to differences in study design (i.e. the duration and dose of *n*-3 PUFA supplementation, choice of control supplement and technique used to measure MPS) and/or participant characteristics. For instance, we observed no differences in p70S6K1 kinase activity or free-living integrated rates of MPS measured over 4 d (assessed by recently re-introduced and less invasive orally administered ^{2}H oxide tracer methodology) between two groups of older adults that combined resistance training with either fish oil (3 g/d) or safflower oil (3 g/d) supplementation[61]. In addition, we demonstrated that 8 weeks of fish oil (5 g/d) derived *n*-3 PUFA (3·5 g/d EPA) did not modulate the 4 h (as measured by the precursor-product method with intravenous infusion of labelled

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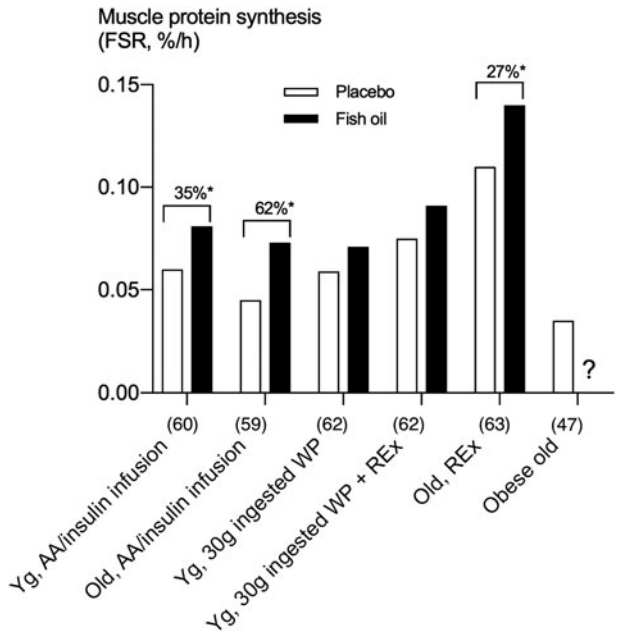

Fig. 3. Overview of findings from experimental studies that investigated the influence of fish oil-derived *n*-3 PUFA supplementation on the response of muscle protein synthesis (MPS) to amino acid provision in young and older adults. Data generated from citations denoted by number in parentheses:[60] young and middle-aged (about 39 years) adults consumed fish oil (4 g/d; 1·86 g/d EPA and 1·50 g/d DHA) capsules over 8 weeks and MPS was measured pre and post supplementation in response to the intravenous infusion of amino acids and insulin.[59] Older (≥65 years) adults consumed fish oil (4 g/d; 1·86 g/d EPA and 1·50 g/d DHA) or maize oil capsules over 8 weeks and MPS was measured in response to the intravenous infusion of amino acids and insulin.[62] Young (about 21 years) adults consumed fish oil (5 g/d; 3·5 g/d EPA and 0·9 g/d DHA) or coconut oil capsules over 8 weeks and MPS was measured in response to ingesting 30 g whey protein at rest and following resistance exercise.[63] Older (65–85 years) adults consumed fish oil (3·9 g/d) capsules over 16 weeks and MPS was measured in response to an acute bout of resistance exercise.[47] MPS was measured in response to ingesting 15 g milk protein isolate in older (66–73 years) obese (BMI > 30) adults. FSR, fractional synthesis rate; Yg, young adults, Old, older adults; AA, amino acid, WP, whey protein; REx, resistance exercise.

phenylalanine) MPS response to ingestion of a 30 g whey protein bolus under both rested and post-exercise conditions in trained young men[62]. Follow-up studies designed with a mechanistic focus are warranted to further explore these findings. We cannot discount the possibility that ingesting 30 g whey protein saturated the muscle protein synthetic machinery in our cohort of 'nutrient-sensitive' trained young men[65], and although more relevant to are warranted to further explore these findings. We can-not discount the possibility that ingesting 30g whey protein saturated the muscle protein synthetic machinery in our cohort of 'nutrient-sensitive' trained young men[65], and although more relevant to simulating daily lifestyle patterns, free-living measurements of MPS integrating postabsorptive and postprandial physiological states might have diluted the chance of detecting any subtle, but physiological relevant, anabolic action of *n*-3 PUFA[61]. Taken together, based on currently available evidence, these data indicate the anabolic action of *n*-3 PUFA may confer greater application to older adults who exhibit a state of anabolic resistance (Fig. 3).

The anabolic action of *n*-3 PUFA in ageing muscle has been partially supported by a series of longitudinal studies that obtained clinically-relevant endpoint measurements of muscle mass, strength and function, particularly when older women were studied. Expanding upon their initial work, Smith *et al.*[4] have demonstrated that daily ingestion of *n*-3 PUFA (1·86 g EPA and 1·50 g DHA) over 6 months increased thigh volume by about 3·5 % and handgrip strength by about 6 % in older adults, despite the absence of structured exercise training. The clinical implications of these remarkable data are particularly significant given that, as mentioned previously, handgrip strength[27] and general strength[66] are known predictors of all-cause mortality. Moreover, we demonstrated that improvements in muscle strength and quality (calculated as peak torque relative to muscle anatomical cross-sectional area), but not muscle mass, following 18 weeks of structured bi-weekly resistance exercise training were augmented with dietary fish oil-derived *n*-3 PUFA supplementation in older women[61]. However, no such benefit of *n*-3 PUFA ingestion was observed when older men were studied. Consistent with this observation, an earlier study supplemented older women with 2 g/d fish oil during 90 d resistance training and reported greater strength gains compared with training alone[5]. However, we contend that these data from Rodacki *et al.*[5] should be treated with caution since no placebo group was included in the study design, the changes in blood *n*-3 PUFA composition were minimal, and no direct measures of muscle mass or MPS were collected. It follows that further studies are warranted to first confirm this apparent sex-difference in the muscle adaptive response to resistance training with *n*-3 PUFA ingestion, and second, determine the mechanism(s) that underpins this apparent sexual dimorphism in response to ingested *n*-3 PUFA.

Accumulating evidence also substantiates a protective role for *n*-3 PUFA ingestion during short-term periods of muscle disuse. In this regard, an elegant recent study by McGlory *et al.*[67] investigated the influence of *n*-3 PUFA supplementation on changes in muscle mass and integrated rates of MPS following 2 weeks of limb immobilisation in young women. The decline in muscle volume elicited by short-term limb immobilisation was attenuated by approximately 6 % with *n*-3 PUFA supplementation (a decrease of 8 %) *v.* the sunflower oil control (a decrease of 14 %). Moreover, following 2 weeks of rehabilitation whereby study participants resumed their habitual physical activity levels, muscle volume returned to baseline levels with *n*-3 PUFA supplementation, but remained below baseline in the control group. Accompanying the retention of muscle volume during simulated muscle disuse atrophy was a higher integrated response of MPS both at the immediate cessation of limb immobilisation and following 2 weeks of remobilisation.

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Interestingly, *n*-3 PUFA supplementation had no protective effect on the decline in muscle strength. Consistent with this observation, albeit using an animal model, rats fed an *n*-3 PUFA-rich diet during hindlimb suspension (simulating leg immobilisation) demonstrated an attenuated loss of muscle mass *v.* rats fed a maize oil-rich diet[68]. Taken together, based on multiple lines of evidence, the preponderance of available data suggests that the optimal diet for maintaining muscle mass with age should consider the dietary intake of *n*-3 PUFA. Future studies are warranted to investigate the impact of *n*-3 PUFA ingestion on age-related changes in body composition in sarcopenic, obese, population groups.

Conclusions

Skeletal muscle plays an underappreciated role in cardiometabolic health and disease. The age-related decline in muscle mass and muscle strength is explained, in part, by the metabolic perturbation termed anabolic resistance. Convincing evidence exists that dietary *n*-3 PUFA ingestion acutely increases the anabolic sensitivity of skeletal muscle in older adults with long-term data indicating a beneficial effect of *n*-3 PUFA ingestion on muscle mass and/or function, particularly in women. Promising, albeit preliminary, evidence suggests that dietary *n*-3 PUFA ingestion may form part of an effective non-pharmacological strategy to attenuate the decline in skeletal muscle mass associated with periods of muscle disuse, e.g. limb immobilisation. Moving forward, larger-scale experimental studies[69] should be repeated in more compromised populations (i.e. frail older adults, sarcopenic obese adults, etc.) to evaluate the application of *n*-3 PUFA ingestion during more extreme periods of muscle disuse, i.e. bedrest during surgery and hospitalisation.

Conflict of Interest

None.

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Authorship

All authors wrote and approved the manuscript.

References

1. Frayn KN (2018) Turning over our fat stores: the key to metabolic health. *Proc Nutr Soc*, [Epublication ahead of print version].
2. Calder PC (2004) n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin Sci* (*Lond*) **107**, 1–11.
3. Di Girolamo FG, Situlin R, Mazzucco S *et al.* (2014) Omega-3 fatty acids and protein metabolism: enhancement of anabolic interventions for sarcopenia. *Curr Opin Clin Nutr Metab Care* **17**, 145–150.
4. Smith GI, Julliand S, Reeds DN *et al.* (2015) Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults. *Am J Clin Nutr* **102**, 115–122.
5. Rodacki CL, Rodacki AL, Pereira G *et al.* (2012) Fish-oil supplementation enhances the effects of strength training in elderly women. *Am J Clin Nutr* **95**, 428–436.
6. Hodson L, Rosqvist F & Parry SA (2019) The influence of dietary fatty acids on liver fat content and metabolism. *Proc Nutr Soc*, [Epublication ahead of print version].
7. Burdge GC & Calder PC (2005) Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* **45**, 581–597.
8. The Scientific Advisory Committee on Nutrition and Committee on Toxicity advice on benefits and risks related to fish consumption (2004) available at https://www.gov.uk/government/publications/sacn-advice-on-fish-consumption (accessed April 2019).
9. Results of the National Diet and Nutrition Survey (NDNS) rolling programme for 2014 to 2015 and 2015 to 2016 (2016) available at https://www.gov.uk/government/statistics/ndns-results-from-years-7-and-8-combined (accessed April 2019).
10. Lentjes MAH, Keogh RH, Welch AA *et al.* (2017) Longitudinal associations between marine omega-3 supplement users and coronary heart disease in a UK population-based cohort. *BMJ Open* **7**. Available at https://www.ncbi.nlm.nih.gov/pubmed/29030414.
11. Albert BB, Derraik JG, Cameron-Smith D *et al.* (2015) Fish oil supplements in New Zealand are highly oxidised and do not meet label content of n-3 PUFA. *Sci Rep* **5**, 7928.
12. Heller M, Gemming L, Tung C *et al.* (2019) Oxidation of fish oil supplements in Australia. *Int J Food Sci Nutr*, **70**, 540–550.
13. Gibbs RA, Rymer C & Givens DI (2010) Postgraduate Symposium: long-chain n-3 PUFA: intakes in the UK and the potential of a chicken meat prototype to increase them. *Proc Nutr Soc* **69**, 144–155.
14. Simopoulos AP (2016) An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients* **8**, 128.
15. Wolfe RR (2006) The underappreciated role of muscle in health and disease. *Am J Clin Nutr* **84**, 475–482.
16. Kim J, Wang Z, Heymsfield SB *et al.* (2002) Total-body skeletal muscle mass: estimation by a new dual-energy X-ray absorptiometry method. *Am J Clin Nutr* **76**, 378–383.
17. Meyer C, Dostou JM, Welle SL *et al.* (2002) Role of human liver, kidney, and skeletal muscle in postprandial glucose homeostasis. *Am J Physiol Endocrinol Metab* **282**, E419–E427.
18. Kotler DP, Tierney AR, Wang J *et al.* (1989) Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. *Am J Clin Nutr* **50**, 444–447.
19. Janssen I, Heymsfield SB, Wang ZM *et al.* (2000) Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J Appl. Physiol (1985)* **89**, 81–88.
20. Mitchell WK, Williams J, Atherton P *et al.* (2012) Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Front Physiol* **3**, 260.
21. Delmonico MJ, Harris TB, Visser M *et al.* (2009) Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *Am J Clin Nutr* **90**, 1579–1585.

22. Goodpaster BH, Park SW, Harris TB *et al.* (2006) The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. *J Gerontol A Biol Sci Med Sci* **61**, 1059–1064.
23. Cruz-Jentoft AJ, Bahat G, Bauer J *et al.* (2019) Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* **48**, 16–31.
24. Landi F, Liperoti R, Fusco D *et al.* (2012) Sarcopenia and mortality among older nursing home residents. *J Am Med Dir Assoc* **13**, 121–126.
25. Janssen I, Heymsfield SB & Ross R (2002) Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* **50**, 889–896.
26. Pinedo-Villanueva R, Westbury LD, Syddall HE *et al.* (2019) Health care costs associated with muscle weakness: a UK population-based estimate. *Calcif Tissue Int* **104**, 137–144.
27. Celis-Morales CA, Welsh P, Lyall DM *et al.* (2018) Associations of grip strength with cardiovascular, respiratory, and cancer outcomes and all cause mortality: prospective cohort study of half a million UK Biobank participants. *Br Med J* **361**, k1651.
28. Hong S, Chang Y, Jung HS *et al.* (2017) Relative muscle mass and the risk of incident type 2 diabetes: a cohort study. *PLoS ONE* **12**, e0188650.
29. Li JJ, Wittert GA, Vincent A *et al.* (2016) Muscle grip strength predicts incident type 2 diabetes: population-based cohort study. *Metabolism* **65**, 883–892.
30. Celis-Morales CA, Petermann F, Hui L *et al.* (2017) Associations between diabetes and both cardiovascular disease and all-cause mortality are modified by grip strength: evidence from UK Biobank, a prospective population-based cohort study. *Diabetes Care* **40**, 1710–1718.
31. Lauby-Secretan B, Scoccianti C, Loomis D *et al.* (2016) Body fatness and cancer – viewpoint of the IARC Working Group. *N Engl J Med* **375**, 794–798.
32. Iliodromiti S, Celis-Morales CA, Lyall DM *et al.* (2018) The impact of confounding on the associations of different adiposity measures with the incidence of cardiovascular disease: a cohort study of 296 535 adults of white European descent. *Eur Heart J* **39**, 1514–1520.
33. Emerging Risk Factors Collaboration, Wormser D, Kaptoge S *et al.* (2011) Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet* **377**, 1085–1095.
34. Atkins JL, Whincup PH, Morris RW *et al.* (2014) Sarcopenic obesity and risk of cardiovascular disease and mortality: a population-based cohort study of older men. *J Am Geriatr Soc* **62**, 253–260.
35. Morley JE (2012) Sarcopenia in the elderly. *Fam Pract* **29** Suppl 1, i44–i48.
36. Markofski MM, Dickinson JM, Drummond MJ *et al.* (2015) Effect of age on basal muscle protein synthesis and mTORC1 signaling in a large cohort of young and older men and women. *Exp Gerontol* **65**, 1–7.
37. Volpi E, Mittendorfer B, Wolf SE *et al.* (1999) Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol* **277**, E513–E520.
38. Cuthbertson D, Smith K, Babraj J *et al.* (2005) Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* **19**, 422–424.
39. Wall BT, Gorissen SH, Pennings B *et al.* (2015) Aging is accompanied by a blunted muscle protein synthetic response to protein ingestion. *PLoS ONE* **10**, e0140903.
40. Guillet C, Prod'homme M, Balage M *et al.* (2004) Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. *FASEB J* **18**, 1586–1587.
41. Balage M, Averous J, Remond D *et al.* (2010) Presence of low-grade inflammation impaired postprandial stimulation of muscle protein synthesis in old rats. *J Nutr Biochem* **21**, 325–331.
42. Wall BT, Snijders T, Senden JM *et al.* (2013) Disuse impairs the muscle protein synthetic response to protein ingestion in healthy men. *J Clin Endocrinol Metab* **98**, 4872–4881.
43. Bell KE, von Allmen MT, Devries MC *et al.* (2016) Muscle disuse as a pivotal problem in sarcopenia-related muscle loss and dysfunction. *J Frailty Aging* **5**, 33–41.
44. Breen L, Stokes KA, Churchward-Venne TA *et al.* (2013) Two weeks of reduced activity decreases leg lean mass and induces "anabolic resistance" of myofibrillar protein synthesis in healthy elderly. *J Clin Endocrinol Metab* **98**, 2604–2612.
45. Wall BT, Dirks ML, Snijders T *et al.* (2014) Substantial skeletal muscle loss occurs during only 5 days of disuse. *Acta Physiol* (*Oxf*) **210**, 600–611.
46. Ferrando AA, Lane HW, Stuart CA *et al.* (1996) Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am J Physiol* **270**, E627–E633.
47. Smeuninx B, Mckendry J, Wilson D *et al.* (2017) Age-related anabolic resistance of myofibrillar protein synthesis is exacerbated in obese inactive individuals. *J Clin Endocrinol Metab* **102**, 3535–3545.
48. Stephens FB, Chee C, Wall BT *et al.* (2015) Lipid-induced insulin resistance is associated with an impaired skeletal muscle protein synthetic response to amino acid ingestion in healthy young men. *Diabetes* **64**, 1615–1620.
49. Calder PC (2006) n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* **83**, 1505S–1519S.
50. Kamolrat T & Gray SR (2013) The effect of eicosapentaenoic and docosahexaenoic acid on protein synthesis and breakdown in murine C2C12 myotubes. *Biochem Biophys Res Commun* **432**, 593–598.
51. McGlory C, Galloway SD, Hamilton DL *et al.* (2014) Temporal changes in human skeletal muscle and blood lipid composition with fish oil supplementation. *Prostaglandins Leukot Essent Fatty Acids* **90**, 199–206.
52. Calder PC (2010) Omega-3 fatty acids and inflammatory processes. *Nutrients* **2**, 355–374.
53. Rees D, Miles EA, Banerjee T *et al.* (2006) Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. *Am J Clin Nutr* **83**, 331–342.
54. Ebbesson SO, Ebbesson LO, Swenson M *et al.* (2005) A successful diabetes prevention study in Eskimos: the Alaska Siberia project. *Int J Circumpolar Health* **64**, 409–424.
55. Bang HO, Dyerberg J & Sinclair HM (1980) The composition of the Eskimo food in north western Greenland. *Am J Clin Nutr* **33**, 2657–2661.
56. Abdelhamid AS, Brown TJ, Brainard JS *et al.* (2018) Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev* **11**, CD003177.
57. Greenberg JA, Bell SJ & Ausdal WV (2008) Omega-3 fatty acid supplementation during pregnancy. *Rev Obstet Gynecol* **1**, 162–169.
58. Robinson SM, Jameson KA, Batelaan SF *et al.* (2008) Diet and its relationship with grip strength in community-

dwelling older men and women: the Hertfordshire cohort study. *J Am Geriatr Soc* **56**, 84–90.
59. Smith GI, Atherton P, Reeds DN *et al.* (2011) Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: a randomized controlled trial. *Am J Clin Nutr* **93**, 402–412.
60. Smith GI, Atherton P, Reeds DN *et al.* (2011) Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. *Clin Sci* **121**, 267–278.
61. Da Boit M, Sibson R, Sivasubramaniam S *et al.* (2017) Sex differences in the effect of fish-oil supplementation on the adaptive response to resistance exercise training in older people: a randomized controlled trial. *Am J Clin Nutr* **105**, 151–158.
62. McGlory C, Wardle SL, Macnaughton LS *et al.* (2016) Fish oil supplementation suppresses resistance exercise and feeding-induced increases in anabolic signaling without affecting myofibrillar protein synthesis in young men. *Physiol Rep* **4**. Available at https://www.ncbi.nlm.nih.gov/pubmed/27009278
63. Lalia AZ, Dasari S, Robinson MM *et al.* (2017) Influence of omega-3 fatty acids on skeletal muscle protein metabolism and mitochondrial bioenergetics in older adults. *Aging* (*Albany NY*) **9**, 1096–1129.
64. Kamolrat T, Gray SR & Thivierge MC (2013) Fish oil positively regulates anabolic signalling alongside an increase in whole-body gluconeogenesis in ageing skeletal muscle. *Eur J Nutr* **52**, 647–657.
65. Witard OC, Jackman SR, Breen L *et al.* (2014) Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am J Clin Nutr* **99**, 86–95.
66. Metter EJ, Talbot LA, Schrager M *et al.* (2002) Skeletal muscle strength as a predictor of all-cause mortality in healthy men. *J Gerontol A Biol Sci Med Sci* **57**, B359–B365.
67. McGlory C, Gorissen SHM, Kamal M *et al.* (2019) Omega-3 fatty acid supplementation attenuates skeletal muscle disuse atrophy during two weeks of unilateral leg immobilization in healthy young women. *FASEB J* **33**, 4586–4597.
68. You JS, Park MN, Song W *et al.* (2010) Dietary fish oil alleviates soleus atrophy during immobilization in association with Akt signaling to p70s6k and E3 ubiquitin ligases in rats. *Appl Physiol Nutr Metab* **35**, 310–318.
69. Pahor M, Anton SD, Beavers DP *et al.* (2018) Effect of losartan and fish oil on plasma IL-6 and mobility in older persons. The ENRGISE Pilot randomized clinical trial. *J Gerontol A Biol Sci Med Sci*. Available at https://www.ncbi.nlm.nih.gov/pubmed/30541065
70. McCance and Widdowson's 'composition of foods integrated dataset' on the nutrient content of the UK food supply (2019) available at https://www.gov.uk/government/publications/composition-of-foods-integrated-dataset-cofid (accessed April 2019).
71. Yang Y, Breen L, Burd NA *et al.* (2012) Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br J Nutr* **108**, 1780–1788.
72. Yang Y, Churchward-Venne TA, Burd NA *et al.* (2012) Myofibrillar protein synthesis following ingestion of soy protein isolate at rest and after resistance exercise in elderly men. *Nutr Metab* (*Lond*) **9**, 57.
73. Beals JW, Sukiennik RA, Nallabelli J *et al.* (2016) Anabolic sensitivity of postprandial muscle protein synthesis to the ingestion of a protein-dense food is reduced in overweight and obese young adults. *Am J Clin Nutr* **104**, 1014–1022.